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MOLECULAR DOCKING SIMULATION STUDY OF PHYTOESTROGENS FROM *ASPARAGUS RACEMOSUS* IN BREAST CANCER PROGRESSION

Ramit Singla and Vikas Jaitak*

Centre for Chemical and Pharmaceutical Sciences, School of Basics and Applied Sciences Central University of Punjab, Bathinda (Punjab)-151001 India.

Keywords:
Oestrogen receptors; glucose-6-phosphate dehydrogenase; HSP90; steroid sulphatase; tubulin; 17β-hydroxysteroid dehydrogenase; *in-silico*; phytoestrogens

Correspondence to Author:
Vikas Jaitak
Assistant Professor
Centre for Chemical and Pharmaceutical Sciences School of Basics and Applied Science Central University of Punjab, Bathinda (Pb), India-151001, India

E-mail: vikasjaitak@gmail.com

ABSTRACT: Phytoestrogens are the xenoestrogens which are derived from the plant. This is the first report providing the deeper inside into the mechanism involved in restricting breast cancer progression with the help of docking simulation by phytoestrogen. Docking experiment bring to light that phytoestrogens prevents the binding of oestradiol with its receptor, thereby down regulating the signalling pathway and also acts as inhibitor of enzymes involved in the biosynthesis of endogenous oestradiol. In the present study, thirty phytoestrogen reported from *A. racemosus* were selected as ligand along with reference compounds using Maestro 9.3. Rutin, shatavarin I, 3, 6, 4' - trimethoxy-7-O-β-D-glucopyranosyl [1→4]-O-α-D-xylopyranoside glucopyranpsyl, 8-methoxy-5,6,4-trihydroxyisoflavone-7-O-β-D-glucopyranoside,shatavarin X, racemoside A, immunoside showed better interactions with their targets indicated from their respective dock score.

INTRODUCTION: Breast cancer (BC) is the most common type of cancers prevalent in women; 1.67 million new cancer cases have been diagnosed in 2012. Majority of breast tumours initially are hormone-responsive with circulating oestrogens play a vital role in their growth. Two approaches have been reported for managing hormone-dependent breast cancer (HDBC), one is to prevent the binding of oestrogen to its cognate receptor and other is to inhibit its biosynthesis [1]. Phytoestrogens (PE's) are the xenoestrogens, which are derived from the plant and also referred as "dietary oestrogens".

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Studies carried out indicated that the dietary intake of PE's reduced the incidence of BC[2, 3]. Genestein, an isolflavone which is regarded as PE's at higher doses reduced the proliferation of MCF-7 (Breast cancer cell line) cell lines [4]. The mechanism behind the anti-proliferative activity of PE's is unknown *Asparagus racemosus* is an ayurvedic plant belonging to family liliacea that contain phytochemicals which are oestrogenic in nature and called as PE's [5, 6].

A. racemosus root extract was shown to have a protective effect in the mammary cell carcinoma [6]. Steroidal components of *A. racemosus* were also investigated for the apoptotic activity and inferred to have capacity for causing tumor cell death [7]. Anticancer activity of shatavarins which were isolated from the roots have been evaluated by MTT assay using MCF-7 (human breast cancer), HT-29 (human colon adenocarcinoma), A-498 (human kidney carcinoma) cell lines and *in vivo*

experimental model of Ehrlich ascites carcinoma (EAC) tumor bearing mice. Experimental results indicated that, extract (containing shatavarin IV) possesses potent anti-cancer activity [8].

In the present study with the aid of molecular docking simulation, using Maestro software 9.3 [9] the mechanism behind the anti-proliferative activity of thirty PE's from *A. racemosus* [10-15] is determine.

The current docking study revealed that the PE's have a multiple targeted approach for the inhibition of BC proliferation. The targets selected for the study were ERβ (Oestrogen receptor-β), ERα (Oestrogen receptor-α), HSP90 (Heat shock protein) protein, steroid sulphatase/human placental estrone sulphatase, glucose-6-phosphate dehydrogenase, 17β-hydroxy dehydrogenase and colchicine binding site of tubulin protein (**Fig. 1**).

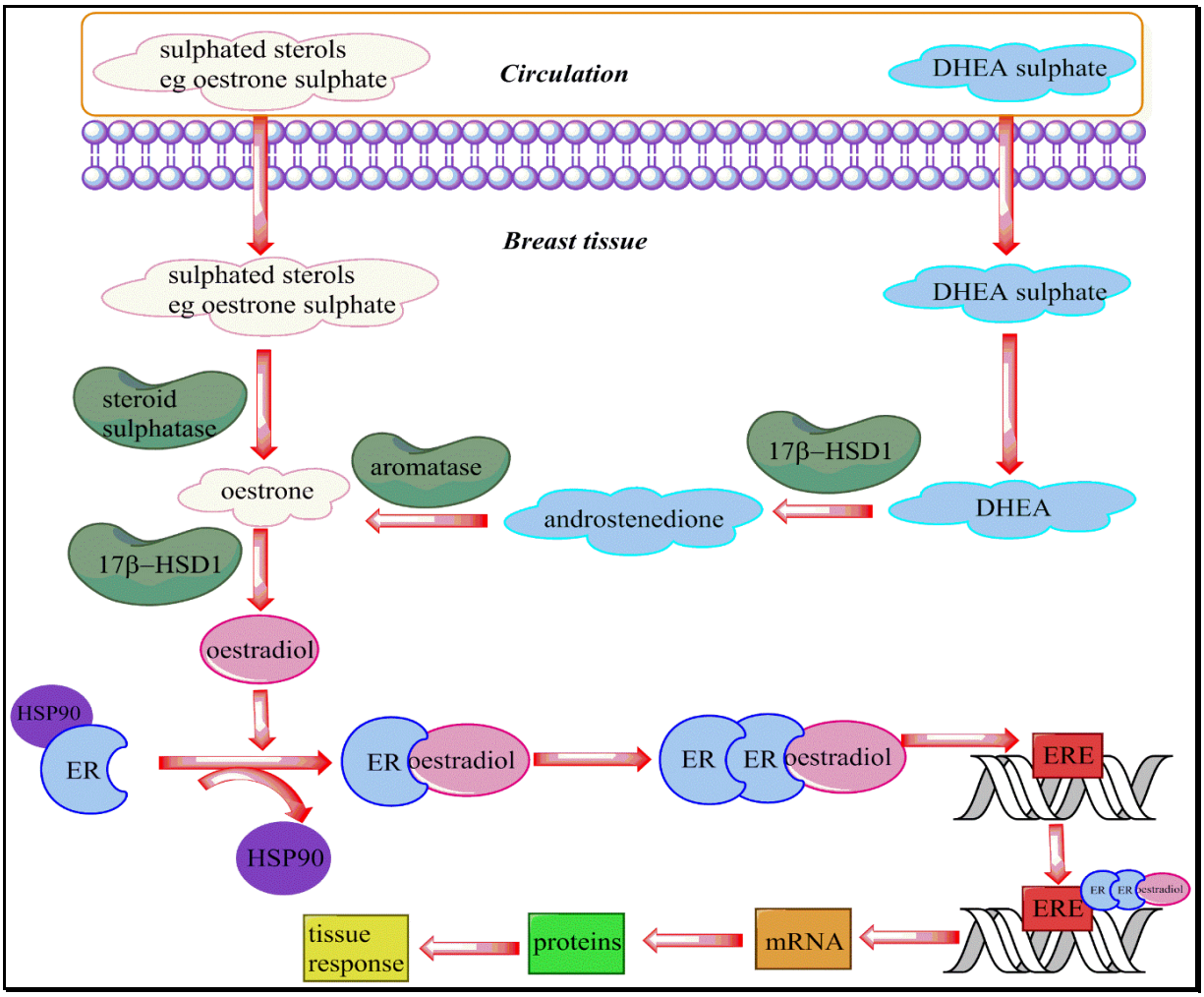


FIG. 1: OESTRADIOL BIOSYNTHESIS PATHWAY AND IT'S BINDING TO THE ER

MATERIAL AND METHOD:

Computational analyses were carried out on windows 7 professional platform running on an HP-Work Station K800 series with Intel Xeon processor and 8 GB of RAM. Molecular docking simulation study was utilized to determine possible binding modes of a ligand to the active site of a receptor. Docking studies has been performed with a set of reported thirty PE's from *A.racemosus* using Maestro 9.0 on ERβ ligand binding domain (3OLS) [15], ERα ligand binding domain (3ERT) [16], HSP90 (1YET) [17], Human placental estrone sulphatases (1P49) [18], human 17β-hydroxysteroid dehydrogenase type 1(1FDS) [19], human glucose 6-phosphate dehydrogenase (2BH9) [20], tubulin protein (1SA0) [21]. The X-Ray structures of proteins were retrieved from the RCSB (http://www.rcsb.org).

Ligand preparation:

Structures of PE's from *A. racemosus* were retrieved from the literature survey (**Fig. 2**)[10-15]. Ligands used were sketched by using maestro 9.3

and converted to 3D structure from 2D using "LigPrep" version 2.5 [9]. "LigPrep" produces a single, low energy, 3D structure with correct chiralities for each input structure. During the performance of this step, chiralities were determined from 3D structure and original states of ionization were retained. "Ligprep" application of the Maestro 9.3 utilizes OPLS-2005 force field.

Protein preparation:

The PDB for the crystal structure of ERβ ligand binding domain (3OLS) [15], ERα ligand binding domain (3ERT) [16], HSP90 (1YET) [17], human placental estrone sulphatases (1P49) [18], human 17β-hydroxysteroid dehydrogenase type 1(1FDS) [19], human glucose 6-phosphate dehydrogenase (2BH9) [20], tubulin protein (1SA0) [21] were retrieved from the RCSB. Protein structure with polar hydrogen was prepared using the protein preparation wizard in Maestro 9.3 [9].

In this step, bond orders were assigned, all hydrogen were added, and bonds to metals were deleted and formal charges were set on the metal and the neighbouring atoms and water molecules were deleted that were more than the 5 Å specific distance. Any missing disulphide bonds were added. The H-bonds were optimized using "protassingn" at pH 7. With generated Het states options, prediction of ionization, and tautomeric states of the het group at pH 7 was achieved.

In protein preparation, reorienting hydroxyl group, water molecules, and amino acids lead to the optimization of hydrogen bond network. Refinement of the structure was the final step in the protein preparation, with the help of restrained minimization. It was initiated in the imperfect minimization with the 0.3 Å RMSD for the minimization OPLS-2005 force field. All bound ligands (small molecules and BH3 peptides), waters beyond 5 Å and ions, molecules and heteroatoms were removed from the complexes [22].

Molecular Docking Simulation:

For the determination of the druggable pocket of protein human glucose 6-phosphate dehydrogenase, human placental estrone sulphatases for which the ligand is not available in the co-crystal structure, "SiteMap" module of Schrodinger was utilized. "Sitemap" provides an efficient way for the determination of ligand binding in 3D structural format. The modules perform calculations on the whole protein to locate binding sites whose size, functionality, and extent of solvent exposure meet user specific criterion. Default parameters were taken for the sitemap, which included use of more restrictive definition of hydrophobicity and the use of standard grid. OPLS-2005 force field was used. Five sites were generated the top ranked site was selected on the basis of Sitescore, size, D Score and volume for grid generation [9]. Ligand molecule was picked for the grid generation, and is excluded from the grid generation. The grid was generated in which Van der Waals scalling was reduced to 0.20 for the ERβ, ERα and 0.50 for the HSP90, human placental estrone sulphatase, human 17β-hydroxysteroid dehydrogenase type 1, human glucose 6-phosphate dehydrogenase and colchicine binding site of the tubulin to soften the potential for non-polar parts of the receptor with partial atomic charge cutoff of 0.25.

The length of the ligands to be docked was increased to 36 Å. The X, Y, Z-ranges were 46, 46, 46 respectively. Glide is a combination of tools which searches for the possible favourable interactions between ligand molecule and receptor molecule. More accurate scoring of the ligand possess is achieved by the Grid which represents the shape and properties of the receptor with the help of several different sets of field. Docking of the reported PE's and the inhibitor was done with XP (extra precision), XP descriptors. Ligand was taken as flexible. Sample nitrogen inversions and sample ring conformations were taken into account. Bias sampling of torsions was one only for the amides and non-polar conformations were penalized.

Epik penalties were added to the docking score. Van der Waals scalling was taken as 0.20 for ERβ, ERα; 0.50 for HSP90, human placental estrone sulphate, glucose-6-phosphate dehydrogenase and tubulin protein; 0.80 for 17β-hydroxysteroid dehydrogenase type I and the partial charge cutoff was taken 0.15 to soften the non-polar parts of the ligand. 10000 poses per docking run were allowed to be run and 1 pose per ligand was allowed to be written. In the post docking minimization number

of poses per ligand to be included was taken to be 10. The threshold energy below which the pose to be rejected was 0.5 kcal/mol [9].

RESULTS AND DISCUSSION:

In continuous to our work on *A. racemosus* [23], we herein with report on *in silico* study of PE's for the treatment of BC. Previous literature indicated that extracts have a protective effect [6] and apoptotic potential [7] on the mammary cell carcinoma. The antiproliferative activity of Shatavarins which was determined by MTT assay using MCF-7, A-498 cell lines and *in-vivo* experimental model of Ehrlich ascites carcinoma (EAC) [8] further strengthen the earlier findings. But the mechanism and the receptor involved in the impressive anti-cancer activity were unknown.

The current molecular docking simulation study suggested that the PE's from *A. racemosus* have a multiple targeted approach, leading to the protection against BC.

Top scoring PE's and their Lipophilic EvdW, H_{bond} value, residues involved in H-bonding, π-π stacking obtained upon docking over ERβ, ERα, HSP90, human steroid sulphatase (human placental estrone sulphatase), 17β-hydroxysteroid dehydrogenase, glucose-6-phosphate dehydrogenase, tubulin protein and along with the dock score of their respective inhibitors; oestradiol, tamoxifen [24, 25] geldanamycin [26], KW-2581[27], estra-1,3,5(10)-triene – 16 - acetamide, 3-hydroxy-17-oxo-*N*-(3-pyridinylmethyl)-,(16β)-methyl, DHEA and 2-methoxy oestradiol respectively [28-30] are given in **Table 1**.

TABLE 1: DOCKSCORE, LIPOPHILICEVDW, HBOND VALUE, RESIDUES IN H-BONDING AND Π-Π STACKING OF PHYTOESTROGENS AND STANDARDS UPON DOCKING OVER RESPECTIVE RECEPTORS.

Sl.No	Receptor	Molecule	Dockscore (kcal/mol)	Lipophilic Evd W	H Bond	Residues in H-bonding and π-π stacking
1	ERβ (3OLS)	Rutin	-11.01	-1.52	-4.59	Leu 301, Glu 305 (**Fig. 2a**)
		Oestradiol	-0.14	-0.68	0	*Phe 356* (**Fig. 2b**)
2	ERα (3ERT)	Shatavarin I	-10.7	-0.7	-7.96	ASP 351, ASN 519, LEU 525 (**Fig. 3a**)
		Tamoxifen	-3.54	-2.01	0	-(**Fig. 3b**)
3	HSP90 (1YET)	3,6,4'-trimethoxy-7-O-β-D-glucopyranosyl [1→4]-O-α-D-xylopyranoside glucopyranpsyl	-12.01	-2.7	-4.21	Asp 93, Thr 115 , *Tyr 139* (**Fig. 4a)**
		Geldanamycin	-3.74	-1.19	-1.05	Asp 102 , Lys 112 (**Fig. 4b**)
4	Human placental Estrone sulphatase (1P49)	8-Methoxy-5,6,4-trihydroxyisoflavone-7-O-β-D-glucopyranoside	-11.06	-3.3	-4.81	Arg 98, Val 101, Trp 555, *Phe 233, Phe 553* (**Fig. 5a**)
		KW-2581	-3.45	-1.84	-0.7	Arg 98 (**Fig. 5b**)
5	17β-hydroxysteroid dehydrogenase type 1 (1FDS)	Shatavarin X	-14.15	-5.34	-6.7	Ile 14, Asn 90, Tyr 155, Thr 190, Val 196 (**Fig. 6a**)
		Estra-1,3,5(10)-triene-16-acetamide, 3-hydroxy-17-oxo-*N*-(3-pyridinylmethyl)-, (16β)-methyl	-6.74	-3.73	-1.87	Ser 142, Tyr 155, His 221, *Phe 259* (**Fig. 6b**)
6	Glucose-6-phosphate dehydrogenase (2BH9)	Racemoside A	-11.79	-3.32	-6.6	Asp 42 , Lys 47, Lys 171, Glu 244, Asp 258, Lys 360 (**Fig. 7a**)
		DHEA	-3.25	-1.51	-0.68	Lys 171, Asp 258 (**Fig. 7b**)
7	Tubulin protein (1SA0)	Immunoside	-10.92	-2.06	-6.84	Val 238, Val 315, Ala 317, Lys 352 (**Fig. 8a**)
		2-Methoxyestradiol	-6.16	-2.49	-1.17	Val 315 (**Fig. 8b**)

The subscript refers to the residue number. Residues involved in hydrogen bond interactions are shown in boldface, and those involved in π-π stacking are shown with italics.

ER comprised of two subtypes, ERα and ERβ. ERβ, antagonize the growth promoting effect of oestrogen therefore its activation is beneficial in oestrogen sensitive tumors cell [31]. It has been reported that ERβ is significant down regulated in the BC epithelium as compared to the epithelium of normal breast [32] thereby activating specifically this receptor will be the beneficiary in tumor suppression.

PE's have an ability to bind preferentially to the ERβ as compared to ERα [3, 20, 33, 34]. The high dock score of the PE's in comparison to the oestradiol signifies strong binding affinity for ERβ. Rutin with dock score of -11.01kcal/mol have shown hydrogen bond interaction with Glu 305 and Leu 301 whereas standard Oestradiol showed dockscore of -0.14 kcal/mol (**Fig. 2**) with might be due to observed H-bond interaction with the receptor site.

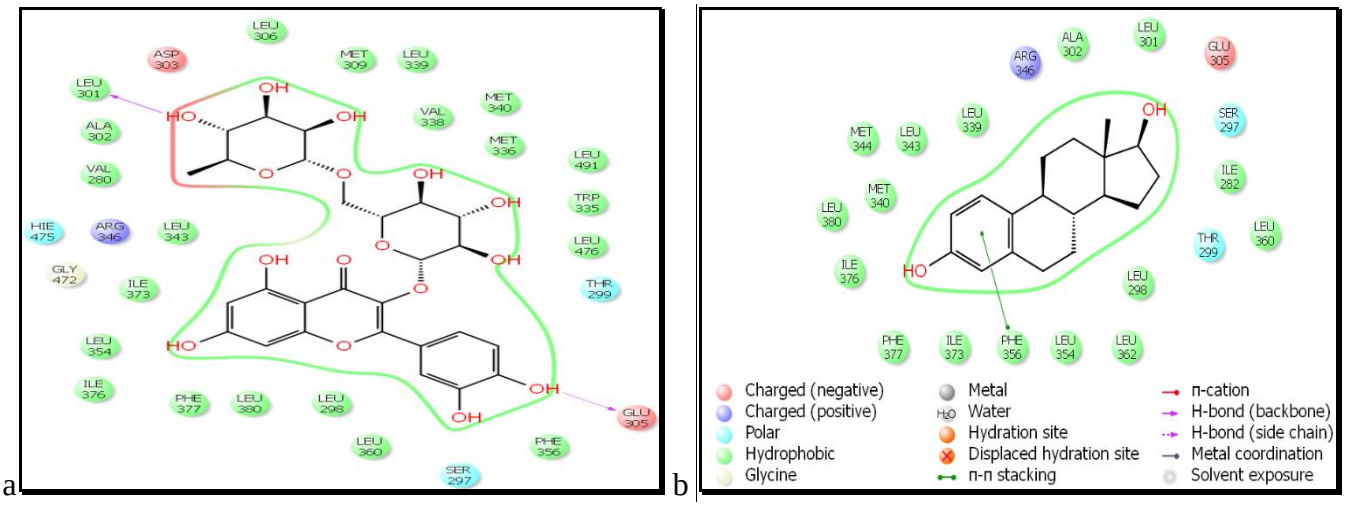


FIG. 2: LIGAND INTERACTION DIAGRAM OF RUTIN (a) AND OESTRADIOL (b) WITH THE ERB (PDP id- 3OLS)

Around 60% of BC's have been detected as ERα positive cancer. ERα plays an indispensable role in the development of mammary gland as well as BC development [35]. ERα controls the transcription of nuclear DNA necessary for human development and important component of BC signalling network thereby emerging as a novel biomarker of the disease. Shatavarin I have been found to possess a dock score of -10.7 kcal/mol and tamoxifen with a dock score of -3.54 kcal/mol **(Fig 3)**. Shatavarin I have showed hydrogen bond interaction with ASP 351, ASN 519 and LEU 525 whereas no H-bonding, exists between tamoxifen and amino acid at receptor site. There is an balance between the ERα and ERβ which regulate the signalling of oestrogen various cellular and biological events of oestrogen-mediated gene regulation in normal and diseased tissues [36]. It has been evidently found that benign breast epithelium have an elevated levels ERα. Higher dock score of PE's in comparison to the tamoxifen implies their strong binding affinity for the receptor indicating a beneficial role in the BC progression.

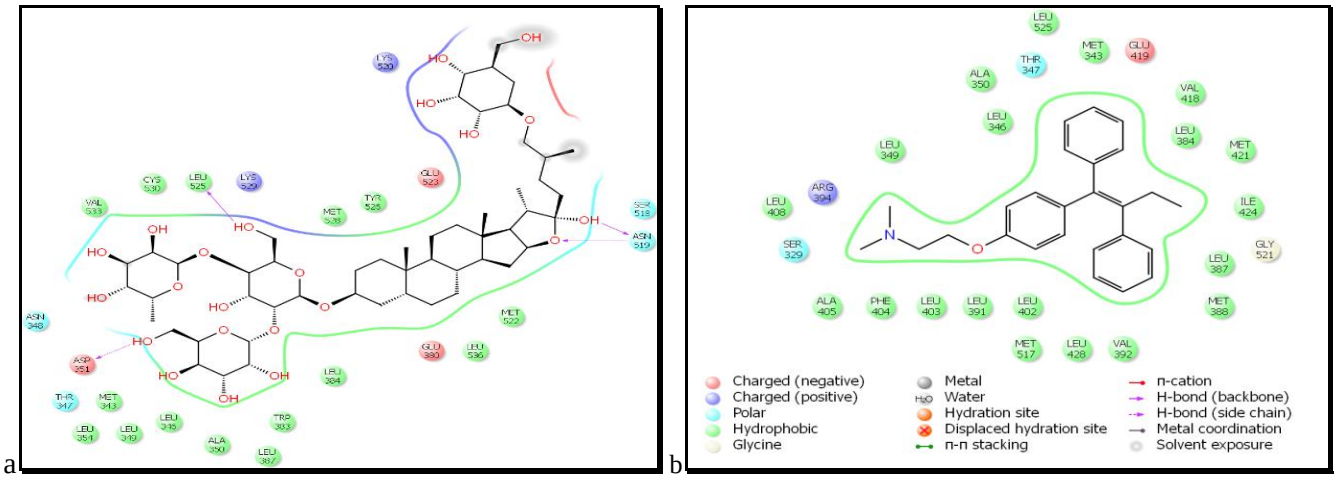


FIG. 3: LIGAND INTERACTION DIAGRAM OF SHATAVARIN I (a) AND TAMOXIFEN (b) WITH THE ERα (PDP id- 3ERT)

ER (α, β) in the inactive state, remains in association with HSP90 [37] a chaperone protein. Once the Ligand oestradiol binds with ER, HSP90 gets dissociated from ER; ER gets dimerised and recognizes a DNA stretch known as ERE (Oestrogen response element).

Thereafter, upon association of the ligand receptor complex with ERE it causes the target gene activation leading to the organization of structural and functional protein essential for the cellular proliferation. HSP90 is found to be up regulated in tumor cells [38]. HSP90 inhibitor geldamycin which is a natural product isolated from fermentation of *Streptomyces hygroscopicus* [39] was noted to decrease the hormone binding to ER [26]. Therefore, inhibitors of HSP90 have potential for decreasing BC proliferation.

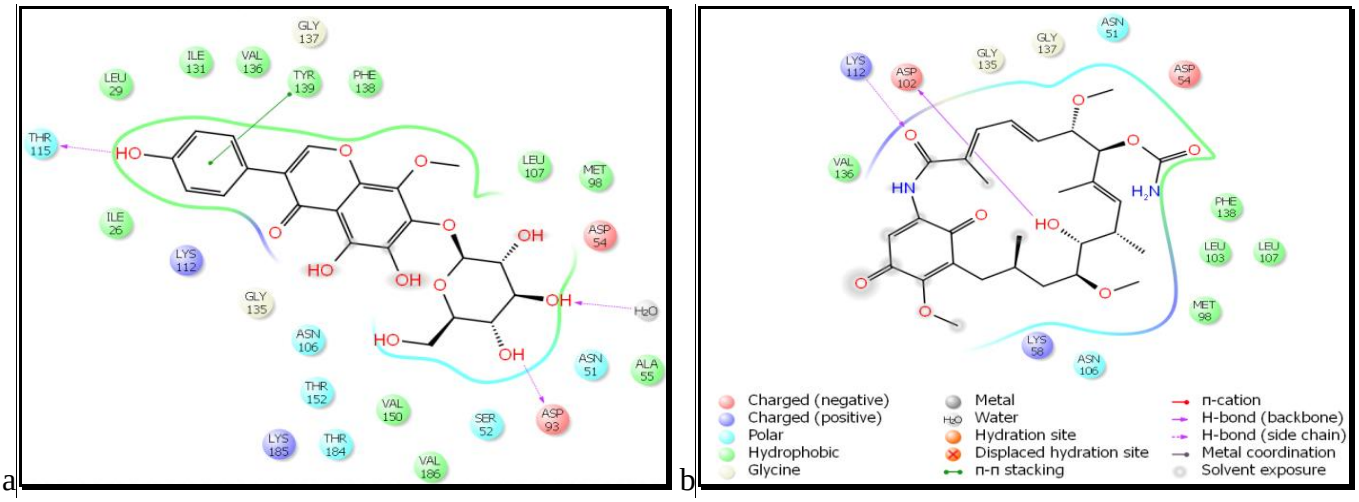


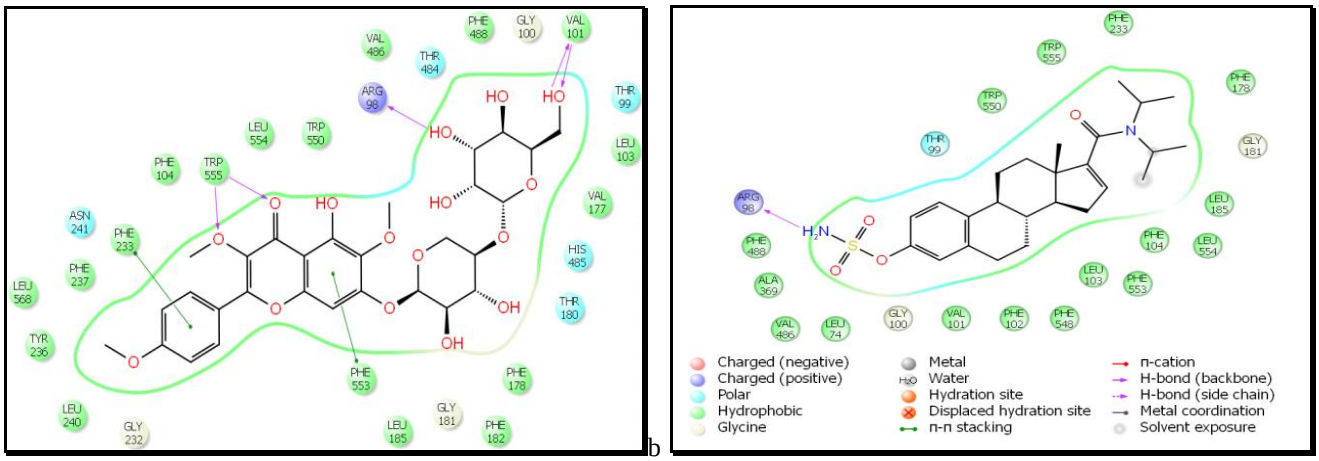
FIG. 4: LIGAND INTERACTION DIAGRAM OF 3,6,4'-TRIMETHOXY-7-O-β-D-GLUCOPYRANOSYL [1→4]-O-α-D-XYLOPYRANOSIDE GLUCOPYRANPSYL (a) AND GELDANAMYCIN (b) WITH THE HSP90 (PDP id- 1YET)

Docking score of the majority of PE's from the *A. racemosus* shows that molecules act as potent inhibitors of HSP90, thus decreasing the downstream signalling initiates upon binding of the oestrogen to ER.

Top scoring molecules 3,6,4'-trimethoxy-7-O-β-D-glucopyranosyl [1→4] – O – α - D -xylopyranoside glucopyranpsyl with docking score of -12.01 kcal/mol have shown an extensive hydrogen bond interaction with Asp 93, Thr 115, and additional van der waals interaction with the receptor site, where as geldanamycin showed docking score of -3.74 kcal/mol (**Fig. 4**); with limited hydrogen bond interaction and no observed van der waals interaction indicating its feeble affinity for the receptor site in comparison to the PE's. sulphatase is an important factor in the steroid dependent BC.

There is an elevated expression of the steroid sulphatases in the BC cells[41]. Steroidal derivative KW-2581 which is an inhibitor of steroid sulphatases [11], decreases the availability of endogenous oestrogen to the cancer cells and thereafter reduces proliferation [41]. Estrone sulphatase is an enzyme that catalyses estrone sulphate into oestrone which is subsequently converted to oestradiol by 17-β-hydroxysteroid dehydrogenase type 1[40]. Oestrone Quercetin, a natural product derivative is found to be an inhibitor of oestrone sulfatase [42].

Impressive dock score and interaction of PE's, 8-methoxy-5,6,4-trihydroxyisoflavone-7-O-β-D glucopyranoside by hydrogen bonding with Arg 98, Val 101, Trp 555 (-11.06 kcal/mol) as compared to the standard drug KW-2581 (-3.45kcal/mol) a limited interaction with the receptor site through hydrogen bonding with Arg 98, indicates that PE's have an inhibitory activity against steroid sulphatase (**Fig. 5**) and thereby decreasing endogenous availability of oestradiol.

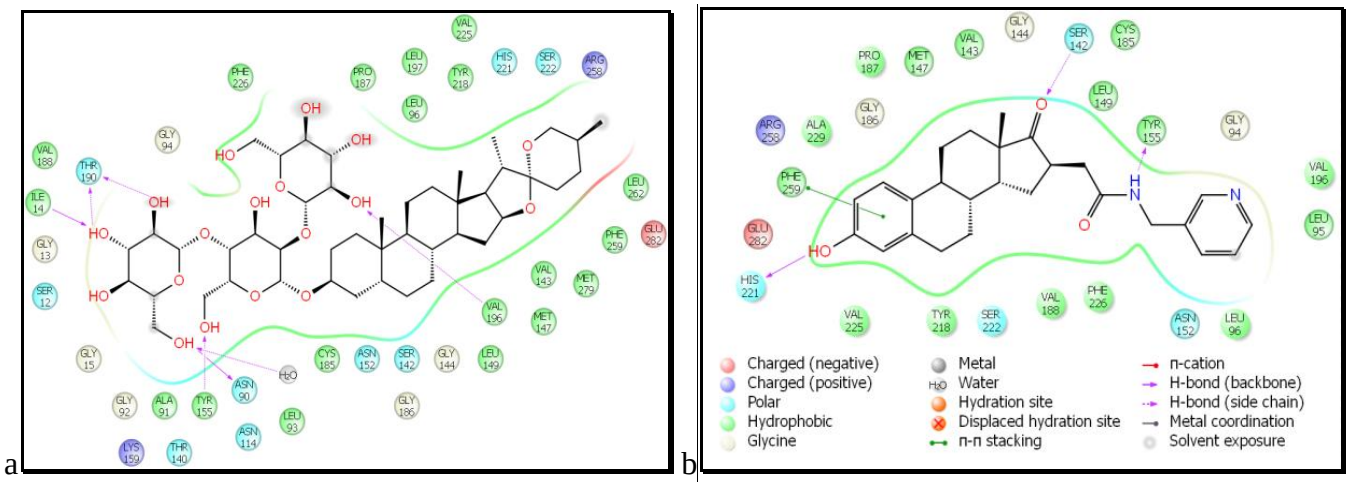


a b

FIG. 5: LIGAND INTERACTION DIAGRAM OF 8-METHOXY-5, 6, 4 - TRIHYDROXYISOFLAVONE-7-O-β-D-GLUCOPYRANOSIDE (a) AND KW-2581 (b) WITH THE HUMAN PLACENTAL ESTRONE SULPHATASE (PDP id- 1P49)

Inactive oestrone is converted into the active oestradiol by the action of 17-β-hydroxysteroid dehydrogenase type I[43]. There is a positive regulator of NM23 anti-metastatic gene on the BC, but 17-β-hydroxysteroid dehydrogenase type 1 increases the migration and stimulated BC growth[44]. Therefore, designing of 17-β-hydroxysteroid dehydrogenase type 1 inhibitors are a striking target for the treatment of HDBC.

Abietic acid, flavanone, 2'-hydroxyflavanone have been shown to have an inhibitory activity on 17-β-hydroxysteroid dehydrogenase type I [45], [46]. Strong binding energy of shatavarin X -14.15kcal/mol as reflected by the dock score due to its hydrogen bond interaction with Ile 14, Asn 90, Tyr 155, Thr 190, Val 196 in comparison to the inhibitor (Estra-1,3,5(10)-triene-16-acetamide, 3-hydroxy-17-oxo-*N*-(3-pyridinylmethyl)-, (16β)-methyl; dockscore -6.74kcal/mol) which only display H-bond interaction with Ser 142, Tyr 155, His 221 signifies their greater binding affinity for the 17β-hydroxysteroid dehydrogenase type 1, thereby decreasing the endogenous availability of the oestradiol by inhibiting its biosynthesis. The interaction profile of shatavarin X and standard is shown in **Fig. 6**.



a b

FIG. 6: LIGAND INTERACTION DIAGRAM OF SHATAVARIN X (a) AND ESTRA-1,3,5(10)-TRIENE-16-ACETAMIDE, 3-HYDROXY-17-OXO-*N*-(3-PYRIDINYLMETHYL)-, (16β)-METHYL (b) WITH THE 17β-HYDROXYSTEROID DEHYDROGENASE TYPE 1 (PDP id- 1FDS)

Glucose-6-phosphate dehydrogenase an enzyme involved in pentose phosphate pathway, responsible for the synthesis of NADPH. Tissues like mammary cells, liver cells, fat and adrenal gland are actively involved in the production of NADPH in the isoprenoid pathway or in the biosynthesis of fatty acids [47]. Genistein and praziquantel from *Flemingia vestita* were shown to possess inhibitory activity on glucose-6-phosphate dehydrogenase [48]. DHEA, a potent inhibitor of

glucose-6-phosphate dehydrogenase is protective in BC [49, 50]. In addition, it was found that there is reduced risk of BC in glucose-6-phosphate deficient women[51]. Dock score of racemoside A (-11.79kcal/mol) due to hydrogen bond interaction with Asp 42, Lys 47, Lys 171, Glu 244, Asp 258 and Lys 360 was found to greater than the standard DHEA (-3.25kcal/mol) suggesting that these PE's strongly binds and inhibits G6PD, similar to the DHEA (**Fig. 7**).

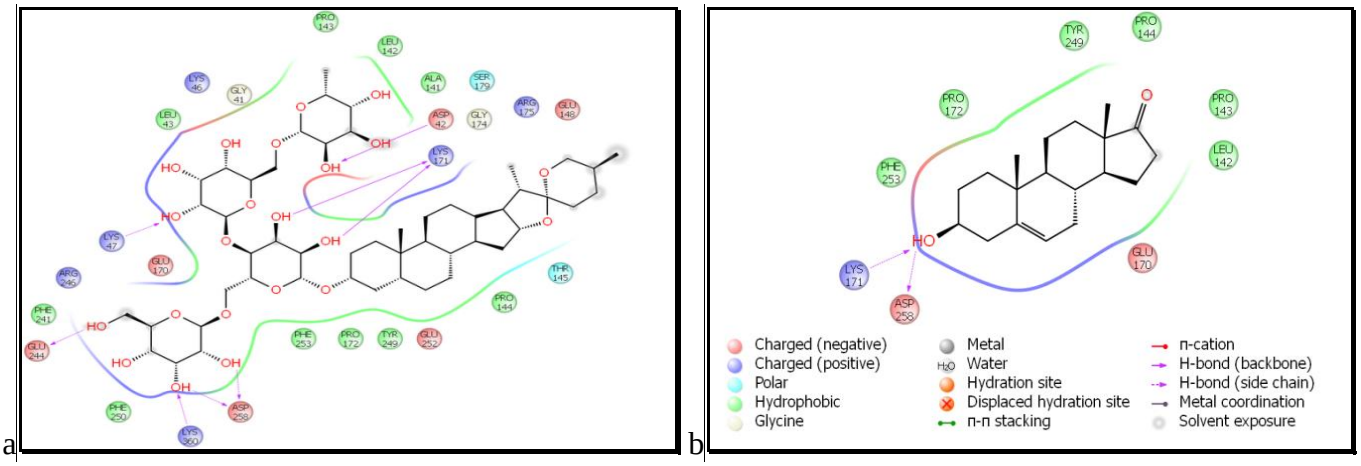


FIG. 7: LIGAND INTERACTION DIAGRAM OF RACEMOSIDE A (a) AND DHEA (b) WITH THE GLUCOSE-6-PHOSPHATE DEHYDROGENASE (PDP id- 2BH9)

Tubulin is the building blocks of the microfilaments [52] required for the chromosome separation during the metaphase of the cell cycle [53]. 2-methoxy oestradiol which is formed upon hydroxylation and methylation of oestradiol endogenously, have a potent anti-proliferative and anti-angiogenic activity, which has been demonstrated in both *in-vivo* and *in-vitro* experiments [54, 55]. 2-methoxy oestradiol has IC_{50} value of 1.4 µM± 0.2 [30] and causes the depolymerisation of the microtubules by binding to the colchicine binding site of the tubulin[56].

ITB-301, glycoside of genistein, has been shown to inhibit the proliferation of SKOV-3 ovarian cancer cells, by acting as a anti-tubulin agent [57].

Docking results suggested that PE's binds to the collagen binding site with a much greater affinity as compared to the standard which is supported by impressive dock score of immunoside; dock score -10.92 kcal/mol resultant of hydrogen bond interaction Val 258, Val 315, Ala 317 and Lys 352 in comparison to the standard 2-methoxyoestradiol **(Fig 8)**. Molecular docking simulation results clearly indicated that rutin, 5-hydroxy 3,6,4'-trimethoxy-7-O-β-D-glucopyranosyl [1→4] -O-α-D-xylopyranoside glucopyranosyl, 8-methoxy-5,6,4-trihydroxyisoflavone-7-O-β-D glucopyranoside, shatavarin X, racemoside A, immunoside were having more binding affinity as compared to their respective standards (**Fig. 8**).

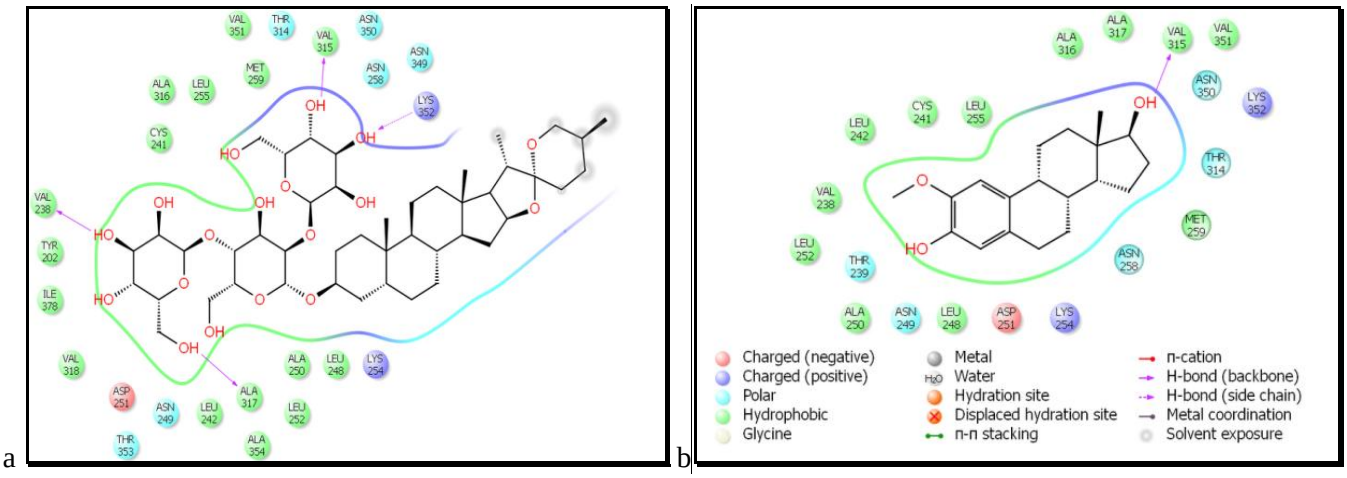


FIG. 8: LIGAND INTERACTION DIAGRAM OF IMMUNOSIDE (a) AND 2-METHOXY ESTRADIOL (b) WITH THE COLCHICINE BINDING SITE OF TUBULIN (PDP id- 1SA0)

The above molecular simulation study on PE's in comparison to the standard drugs indicated that they have strong binding affintiy with the receptors and may be beneficial in the BC treatmnent.

CONCLUSION: Molecular docking simulation study of PE's from *A. racemosus* suggested that, they have a multiple targeted approach for curbing BC proliferation by inhibiting ER down signalling as well as the biosynthesis of oestradiol. PE bind and specifically activate ERβ but at a lower extent as compared to oestradiol thereby acting as a partial agonist; herein, we have demonstrated greater binding affinity of PE's for the receptor as compared to the oestradiol thereby, helpful in tumor suppression.

Activation of ERα has a positive role in managing BC progression; PE's with ERα have shown strong binding affinity which suggests a potential beneficial role in HDBC. Down signalling of ER commences when HSP90 detaches from to ER, but PE's bind to the HSP90 protein and prevent its dissociation from the ER, thereby inhibiting the association of ligand receptor complex with ERE which is required for the target gene activation. PE's also decreases the endogenous availability of the oestradiol by inhibiting biosynthetic pathway.

PE's are known to inhibit 17β-hydroxysteroid dehydrogenase type 1, glucose-6-phosphate dehydrogenase and the docking study also reflected that PE's act as strong inhibitors of the enzyme, thereby decreasing the endogenous availability of oestradiol. The study reckoned that PE's inhibit steroid sulphatase thus, estrone sulphate is not converted to the estrone therefore, decreased availability of the oestrone for the further biosynthesis of oestradiol which leads to a decrease in the endogenous availability of the oestradiol.

Aside from the inhibition of biosynthesis and binding of oestradiol to its receptor, PE's causes the depolymerisation of microfilaments of the tubulin by binding to the colchicine binding site, similar to the 2-methoxyoestradiol and thus helping in tumor suppression. These docking experiments suggest that PE's are the likely candidate for controlling tumor progression with a special emphasis in BC progression. There is further need to perform *in vitro*/*in vivo* bioassays for the establishment of these PE's from the *A. racemosus* in search of the lead in the evolution of cancer chemotherapy.

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CONFLICT OF INTERESTS: The authors have declared no conflict of interests

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